

# PSA and Semenogelin Effectiveness to Detect Semen in Sexual Assault Investigations

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## Background

Semen detection at crime scenes is essential for sexual assault investigations, as it can provide crucial biological evidence linking a suspect to an assault. In these cases, accurate and rapid identification of semen can help confirm the occurrence of sexual contact, guide investigative strategies, and support legal proceedings.

Lateral flow immunochromatographic (LFI) tests targeting prostate-specific antigen (PSA) and semenogelin (Sg) are among the most commonly used tools for presumptive semen detection in forensic workflows. These rapid tests are based on antigen-antibody interactions and provide results within minutes, making them highly valuable in both laboratory and field settings. PSA is produced in the prostate and is present in seminal fluid even in vasectomized individuals, while Sg originates mainly from the seminal vesicles and plays a key role in semen coagulation.

Because of their sensitivity and ease of use, these LFI tests have become essential components of sexual assault evidence processing, enabling fast decision-making and preserving DNA for downstream genetic profiling.

## Objective

The primary objective of this study was to systematically evaluate and compare the sensitivity and specificity of lateral flow immunochromatographic (LFI) assays targeting prostate-specific antigen (PSA) and semenogelin (Sg) across a variety of semen sample types and dilution ranges. By including fresh, pooled, vasectomized, and long-term stored semen samples, we aimed to mimic both recent and cold-case forensic scenarios. In addition, the study sought to determine the practical limits of DNA recovery following immunological testing, assessing whether sufficient DNA quantity and quality could be obtained to enable reliable downstream STR profiling for human identification.

## Methods

Four semen sample types were analyzed to reflect typical and challenging forensic scenarios: fresh single donor, pooled donors, vasectomized donor, and a 30-year-old frozen pool (cold-case simulation). Serial dilutions (1:2 to 1:32,000) were prepared and tested in parallel using SERATEC® PSA Semiquant and RSID™ Semen (Sg) assays, following manufacturer protocols. Sperm presence was confirmed by Christmas Tree staining, allowing differentiation of sperm-positive and sperm-negative samples. DNA was extracted from test membranes and aliquots using the Prepfil kit, quantified with Quantifiler Trio, and evaluated for STR profiling. This approach enabled direct comparison of PSA vs. Sg detection performance across sample types and dilutions, and assessment of DNA recovery potential for downstream forensic analysis.

## Results

### 1. Sensitivity of PSA vs. Semenogelin (Sg)

PSA-based LFI tests remained positive up to a dilution of 1:32,000 across all semen sample types (fresh single donor, pooled donors, vasectomized donor, and 30-year-old frozen pool). In contrast, Sg-based testing showed reduced sensitivity, with a detection limit of approximately 1:8,000 in fresh and pooled samples, and around 1:2,000 in the 30-year-old sample. These findings confirm that PSA provides a more robust signal at higher dilutions, making it particularly suitable for challenging or aged samples.

### 2. Independence from Sperm Presence

PSA positivity was independent of the presence of sperm cells. Even in vasectomized samples and higher dilutions ( $\geq 1:1000$ ), PSA remained positive despite the absence of sperm cells, underscoring its utility in azoospermic or degraded samples.

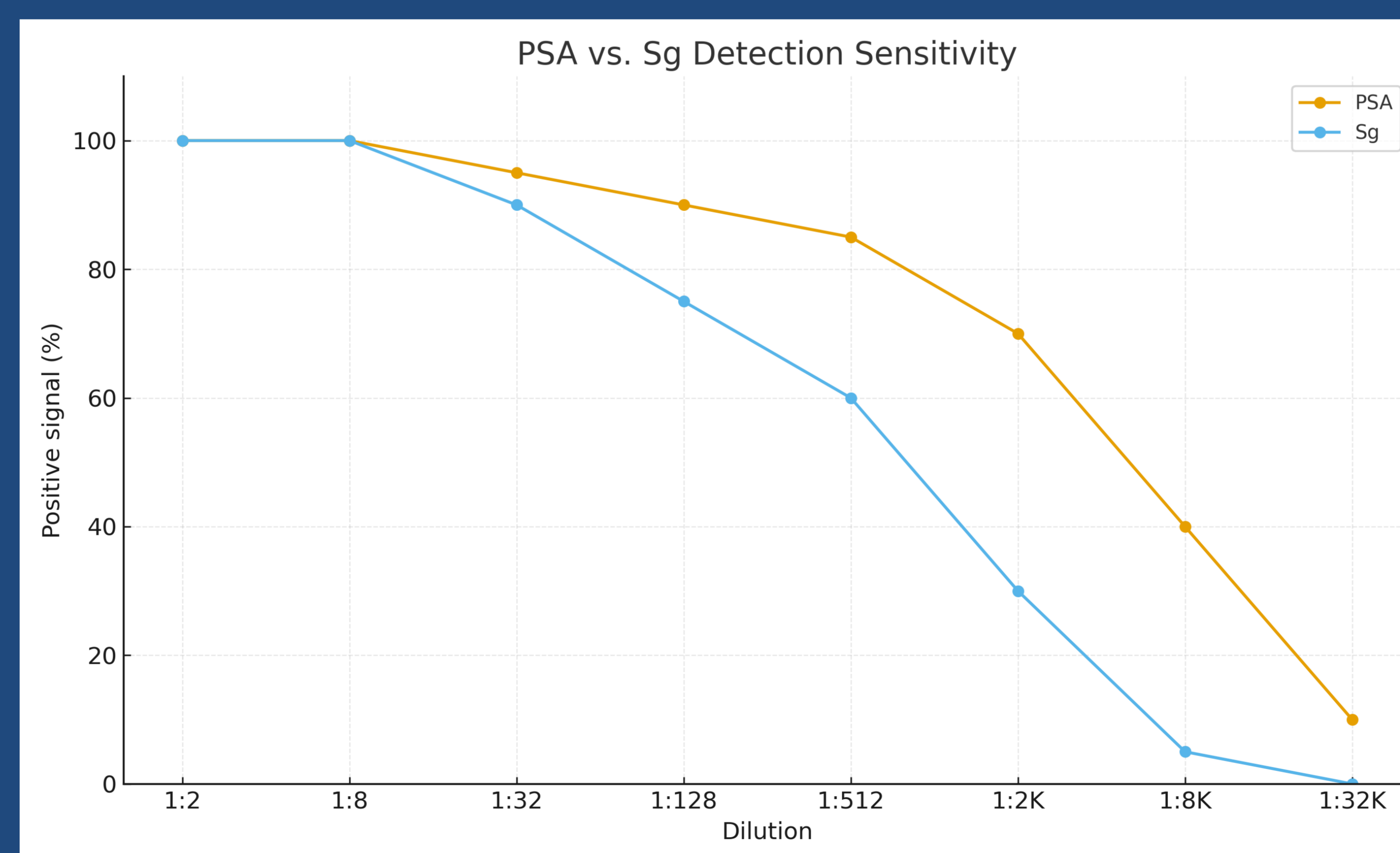
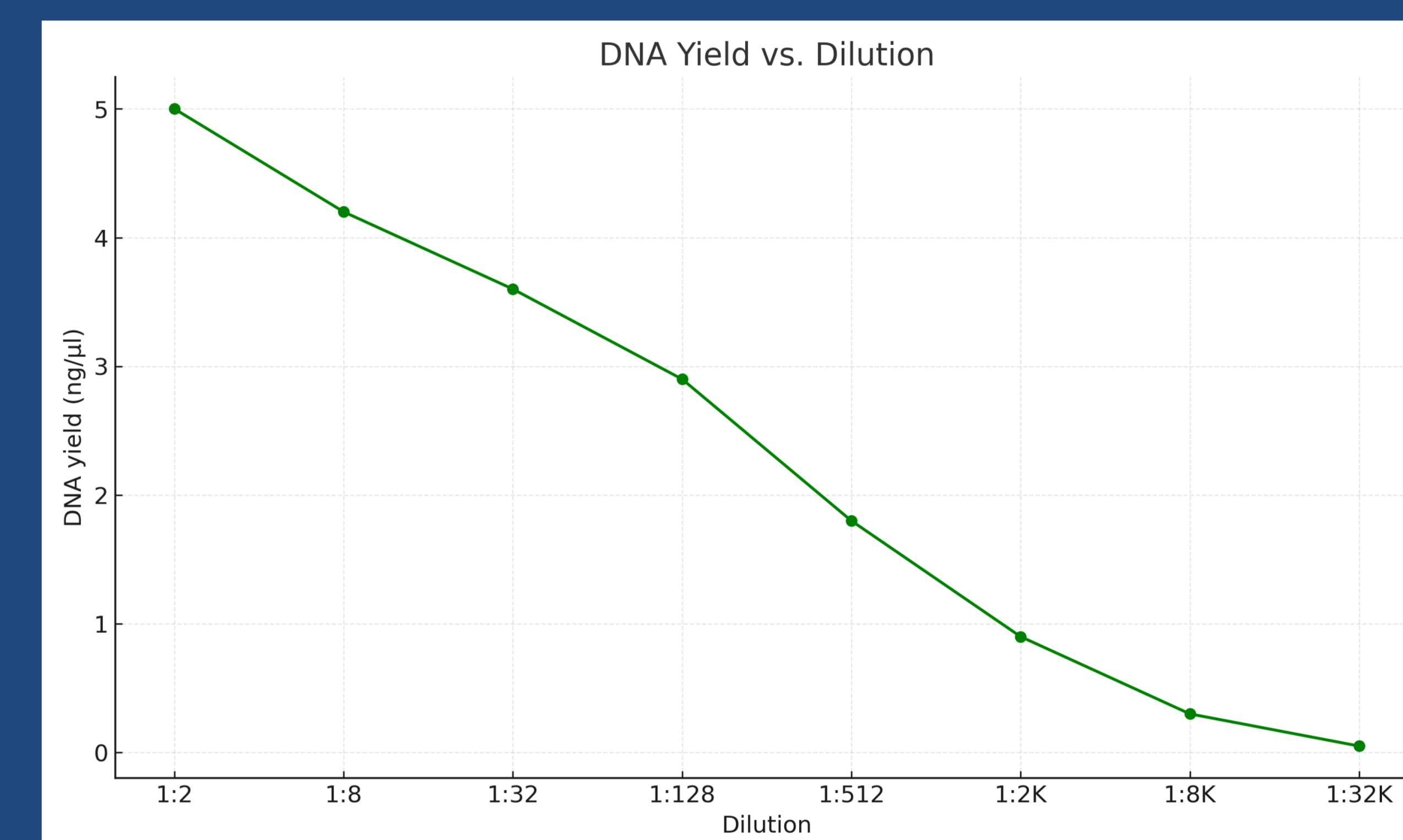
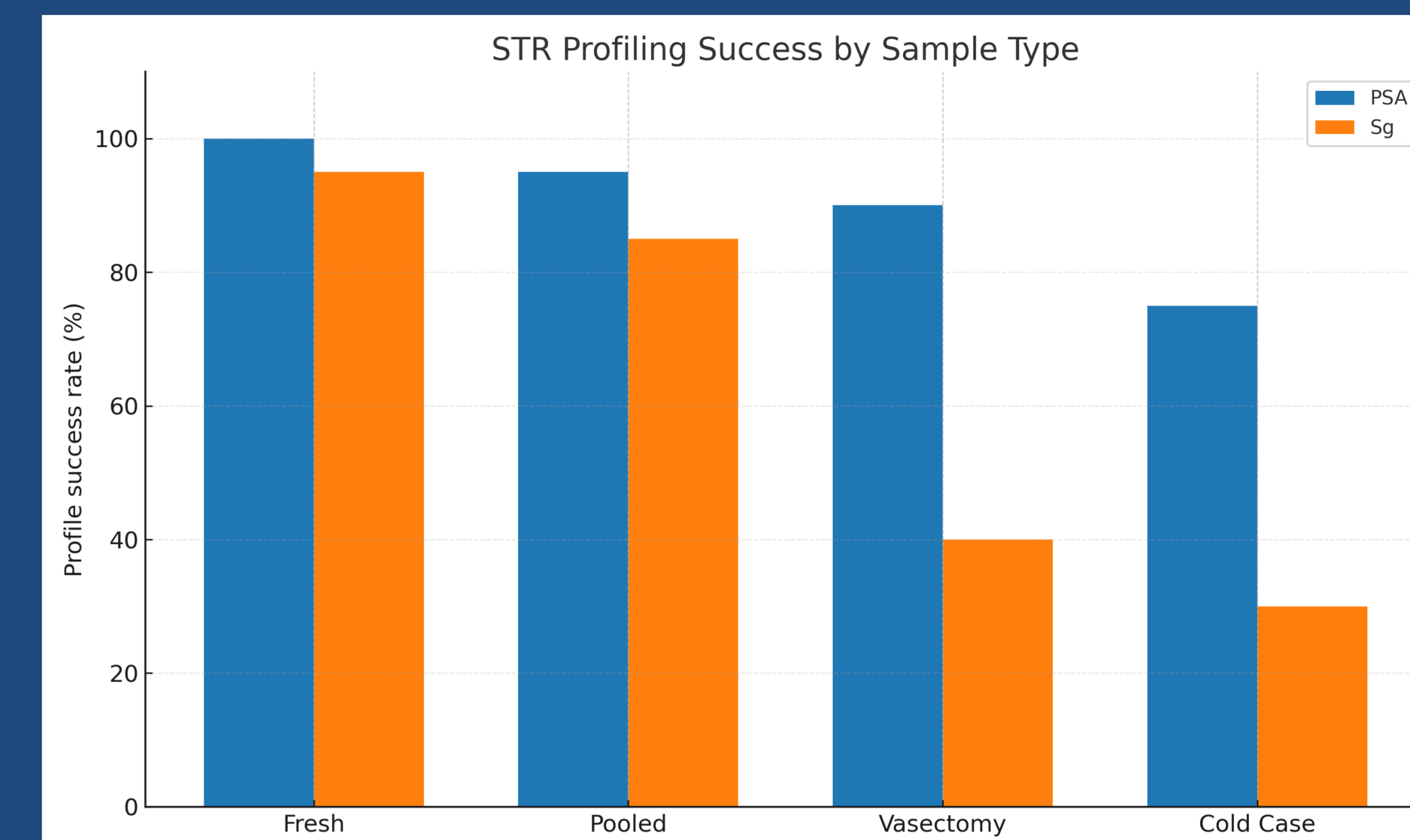
### 3. DNA Recovery

DNA was quantifiable and of sufficient quality for STR and Y-STR profiling up to dilutions of 1:1,000–1:2,000. Beyond this threshold, DNA yield and quality dropped below recommended levels for reliable genotyping, even though PSA remained detectable.

### 4. Cross-reactivity and Specificity

False positives were observed only in a small subset of low-dilution male urine samples, which became negative upon further dilution. All other tested body fluids—including saliva, sweat, breast milk, and most vaginal fluid samples—showed no PSA reactivity, supporting the assay's high specificity.

These results highlight the superior sensitivity and robust performance of PSA-based testing under a variety of forensic conditions, while also emphasizing the importance of confirmatory testing and proper interpretation of results.



## Conclusions

PSA-based LFI tests demonstrated higher sensitivity and more consistent performance than Sg-based assays in the conditions tested, including aged and vasectomized samples. These results indicate that PSA provides a more reliable signal at higher dilutions, making it particularly valuable in challenging or degraded sample scenarios. However, interpretation should still consider factors such as sample quality, storage history, and possible cross-reactivity in certain body fluids. DNA suitable for STR profiling was recoverable up to a dilution of 1:2,000, supporting the combined use of PSA testing and downstream DNA analysis. Limited false positives highlight the importance of appropriate dilution controls and confirmatory testing.